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Microwave Assisted Alcoholysis of Isocyanates Derived from N^{α}-[(9-fluorenylmethyl)oxy]carbonyl Amino Acids: Synthesis of N-Fmoc-N^{1}-Z-/Boc-/Alloc-/Bsmoc-gem-diamines

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A general and efficient method has been developed for the alcoholysis of isocyanates derived from the N^{α}-[(9-fluorenylmethyl)oxy]carbonyl amino acids with various alcohols including hindered ones assisted by MW irradiation. Thus, the synthesis of N, N^{1}-diurethane protected *gem*-diamines wherein Fmoc protection on one of the amino groups and Z-/Boc-/Alloc or Bsmoc group on the other amino function has been accomplished. All the new orthogonally diurethane protected *gem*-diamines have been obtained as crystalline solid powders in 80 to 94% yield. The bisprotected *gem*-diamines have been fully characterized by IR, ^{1}H NMR, ^{13}C NMR as well as by mass spectrometry.

KEY WORDS: alcoholysis; Fmoc-amino acid isocyanates; gem-diamines; microwave irradiation; retro-inverso peptides.

INTRODUCTION

The isocyanates, prepared by the Curtius rearrangement of acyl azides, on alcoholysis gives carbamates which is the basis of the polyurethane industry (Ulrich, 1996). Minakakis and Photaki demonstrated the utility of alcoholysis of isocyanates in the synthesis of N,N^{1}-disubstituted *gem*-diamines possessing Z and ethoxycarbonyl or *p*-methoxybenzyloxy carbonyl groups (Minakakis and Photaki, 1985). However, alcoholysis of isocyanates of highly hindered and sensitive ones to obtain the corresponding cabamates is well known to be difficult. (Claude et al., 2005; Helene and Olivier, 2005). Methanolysis of N-urethane protected isocyanates was found to result in the formation of several side products (Chorev et al., 1984). Further, the reaction of *tert*-butyl alcohol with the isocyanate derived from Z-Leu had not resulted in the desired product. (Cushman et al., 1990). Goodman, Chorev and co-workers, studied side reactions during the synthesis of protected gem-diaminoalkyl derivatives obtained by the curtius rearrangement of acetyl, Boc, or Z-protected phenylalanyl azide, traping the intermediate isocyanates in situ with methanol or benzyl alcohol. They obtained the desired gem-diaminoalkyl derivatives, plus numerous by products. It was concluded that acetyl is better than Z, which is better than Boc, on the basis of maximization of product yield and concurrent minimization of the yield by products. It is reasoned that acetyl protection resulted in fewer by products than carbamate protection due to a reduced tendency of isocyanate to undergo the heterolytic or displacement reactions. (Flecher et al., 1998)

One of the major contributions of Murray Goodman is the synthesis of N-acylated *gem*-diaminoalkyl

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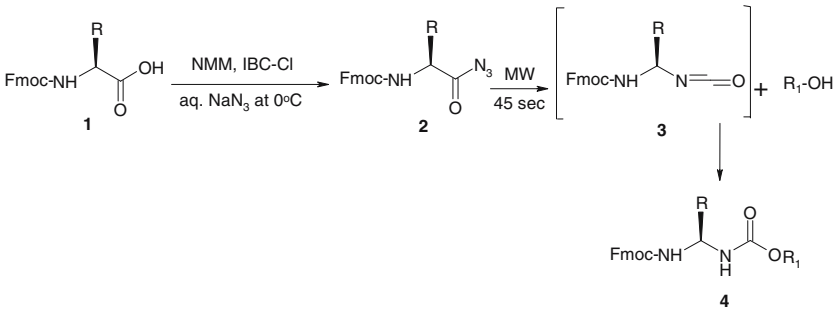


Scheme 1.

Table I. Alcoholysis of Fmoc-Leu-N_3 with benzyl alcohol under various conditions

Entry	Method	Time	Temp (^oC)	Yield (%)
1	Δ	15 h	110	25
2	))))*	12 h	70	35
3	CuCl	60 min	25	40
4	MW	15 min	110	90

* Under ultrasonic conditions

trifluoroacetates by the Hoffmann rearrangement of amides using the mild oxidizing agent iodobenzene bis(trifluoroacetate) (IBTFA) (Chorev et al., 1984; Chorev and Goodman, 1993; Pallai et al., 1983). However, the oxidation reaction using IBTFA employing Fmoc group for N-protection was reported to be ineffective (Englund et al., 2004). As concluded by Scheibler and Chorev, published data suggests that urethane type protecting groups are useful for the synthesis of retro-isomers, retro-inverso isomers, partially modified retro-inverso (PMRIs) isomers, end-group modified peptides, etc. (Flecher et al., 1998; Scheibler and Chorev, 2002). Sureshbabu et al., (Sureshbabu et al., 2000; Vasanthakumar et al., 2002) utilizing the multiple advantages of Fmoc group, (Carpino et al., 1996) reported the preparation, characterization and stability of Fmoc-amino acid azides including side chain protected bifunctional ones and their conversion to the corresponding isocyanates as well as carbamates (Patil and Sureshbabu, 2003; Patil et al., 2003). Both preparation as well as the isocyanates derived from N^{α}-Fmoc-α-amino acids as coupling agents for the synthesis of peptidyl ureas was demonstrated to be completely free from racemization (Patil and Sureshbabu, 2003). This paper demonstrates the synthesis of N-Fmoc-N^1-urethane (Z-/Boc-/Alloc-/Bsmoc) (Carpino et al., 1997, 1999) protected *gem*-diamines and their utility in the synthesis of retro-inverso peptides.

MATERIALS AND METHODS

General

Melting points were taken on a Buchi model 150 melting point apparatus in open capillaries. IR spectra were recorded on a Nicolet model impact 400 D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). ^1H spectra were recorded on a Bruker AMX 300 MHz spectrometer. High resolution mass spectra (HRMS) were recorded on Q-Tof micromass mass spectrometer. Optical rotations were measured on JASCO-DIP-370 digital polarimeter at room temperature. The Sonic bath is German make (35 KHz, Elma, T 310/H). The microwave reaction was carried out in a LG MS 194A microwave oven producing microwave radiation with a frequency of 2450 MHz. The microwave oven and the reaction was speciphically carried out at 60% of the total power output, which would correspond to an average power of 720 W. All the solvents were freshly distilled prior to use.

GENERAL PROCEDURE FOR THE SYNTHESIS OF PROTECTED GEM DIAMINES 4

To the compound 3 (1 mmol) dissolved in dry toluene (20 ml) was added alcohol (3 mmol) in a closed teflon vessel (volume 250 ml) exposed to microwaves for 15 min (4 times, each time 3 min with 5 min intervals) with four intervals. After the completion of the reaction, the remaining solvent was concentrated and then hexane was added. The resulting precipitate was filtered and recrystallized using ethyl acetate: hexane (4:6).

GENERAL PROCEDURE FOR THE SYNTHESIS OF RETRO-INVERSO PEPTIDES 6

A Boc-/Z-amino acid (1 mmol) dissolved in 10 ml of THF was added Fmoc-gemdiamine (1 mmol) in THF (25 ml) and HBTU (1 mmol), DIEA (1.1 mmol) was added to the reaction mixture and

Table II. Microwave assisted synthesis of N-Fmoc-N[1]-Z-/Boc-/Alloc-/Bsmoc-*gem*-diamines

Entry	Substrate	M.P. (^{0}C)	$[\alpha]^{25}_D$ (c = 1, DMSO)	Yield (%)	I.R Mass (cm^{-1})	^{1}H NMR	^{13}C NMR
1	Fmoc-gGly-Z	178	–	81	1705 425.1452	3.3 (2H, m), 4.2 (1H, t), 4.4 (2H, t) 5.08 27.1, 47.0, 48.3, 65.5, (2H, dd),7.25-7.76 (13 H, m).	69.3, 119.9, 123.6, 126.3, 127.2, 127.7, 136.8, 141.6, 149, 157.7, 158.8
2	Fmoc-gAla-Z	140	−122.9	82	1710 439.4667	1.39 (3H, d), 3.9 (1H, m), 4.21 (1H, t), 5.1 (2H, dd), 7.25-7.76 (13H, m)	23.12, 47.0, 60.0, 63.3, 69.3, 119.9, 124, 126.2, 127.2, 127.7, 128.3, 137.4, 141.5, 149,157, 157.5.
3	Fmoc-gVal-Z	194	−57.8	81	1710 467.5253	1.1 (6H, t), 1.7 (1H, m), 3.88 (1H, m), 4.2 (1H, t), 4.4 (2H, t), 7.25-7.76 (13H, m). 127.3	17.3, 28.6, 47.1, 59.6, 69.4,72.7,119.9, 124.4, 126.3, 128.4, 127.8, 136.7, 141.6, 148.8, 156.9, 157.3.
4	Fmoc-gLeu-Z	148	−61.1	90	1715 481.5964	0.91 (6H, t), 1.65 (3H, m), 3.8 (1H, m), 4.17 (1H, t), 4.39 (2H, d), 5.08 (2H, d), 5.6 (1H, br d), 7.31-7.76 (13H, m).	22.2, 22.5, 24.8, 42.9, 47.1, 59.1, 66.6, 66.7, 66.9, 119.9, 24.9, 127.0, 127.5, 128, 128.1 124.8, 128.5, 128.7, 136.2, 141.2, 143.8, 155.4.
5	Fmoc-gIle-Z	198	−78.4	80	1709 481.6701	0.9 (6H, m), 1.13 (1H, m), 1.45 (2H, m), 3.78 (1H, m), 4.17 (1H, t), 4.39 (2H, d), 5.1 (2H, d), 5.6 (1H, br), 7.3- 7.76 (13H, m).	12.7, 25. 6, 32.2, 47.1, 59.7, 69.5, 71.6, 120.0, 124.1, 126.1, 127.1, 127.5, 128.3, 137.4, 141.5, 148.8, 156, 156.1.
6	Fmoc-gPhe-Z	185	−99.4	89	1698 515.6643	2.91 (2H, d), 3.8 (1H, m), 4.17 (1H, t), 4.4 (2H, d), 5.09 (2H, d), 5.68 (1H, br), 7.3-7.76 (18H, m).	40.0, 47.1, 59.6, 68, 69.5, 119. 9, 124.2, 126.3, 127.2, 127.3, 127.8, 128.4, 128.5, 137.4, 144. 6, 148.8, 156.7, 157.0.
7	Fmoc-gPro-Z	120	−17.25	80	1705 465.5321	1.45 (3H, d), 1.86-1.96 (4H, m), 3.3-3.48 (2H, m), 4.14 (1H, m), 4.13 (1H, d), 4.36 (2H, t), 5.06 (2H, s), 5.5 (1H, br), 7.27-7.7 (13H, m)	25.0, 32.4, 47.1, 50.2, 59.3, 67.1, 69.6, 119.9, 124.1, 126.2, 127.1, 127.7, 128.7, 128.9, 137.4, 144.6, 148.8, 156.7, 157.7.
8	Fmoc-gLeu-Alloc	150	−85.45	92	1700 431.5115	0.91 (6H, t), 1.65 (3H, m), 3.8 (1H, m), 4.17 (1H, t), 4.39 (2H, d), 4.55 (2H, d), 5.29 (2H, m), 5.9 (1H, m), 7.24-7.7 (8H, m).	21.9, 23.2, 41.6, 47.0, 6, 66.1, 69.4, 116.3, 119.7, 124.1, 126.2, 127.2, 133.1, 141.8, 148.7, 157.2, 158.1.
9	Fmoc-gPhe-Alloc	168	−187.8	94	1698 465.5867	3.19 (2H, d), 4.2 (1H, t), 4.41 (2H, d), 4.55 (2H, d), 5.29 (2H, m), 5.9 (1H, m), 7.24-7.7 (13H, m).	40.3, 47.2, 66.2, 67.9, 116.3, 120.0, 124.1, 127, 127.2, 126.3, 127.1, 128, 133.2, 130.4, 141.5, 149, 156.2, 157.
10	Fmoc-gAla-Boc	182	−187.52	88	1715 405.4424	1.37 (9H, s), 1.44 (3H, d), 4.24 (1H, t), 4.4 (2H, d), 4.56 (1H, br), 5.29 (1H, br s), 7.3-7.78 (8H, m)	23.1, 28.0, 47.1, 63.2, 70.1, 79.2, 119.9, 124.1, 126.4, 127.2, 141.5, 148.9, 156.0, 157.8.
11	Fmoc-gSer(OBzl)-Boc	202	−50.1	85	1705 511.5788	1.4 (9H, s), 3.6 (2H, d), 3.79, (1H, m), 4.17 (1H, t), 4.39 (2H d), 5.08 (2H, s), 7.25-7.78, (13H, m).	27.8, 47.3, 69.8, 72.2, 73.8, 80.0, 119.5, 124.1, 126.5, 127.3, 127.8, 128, 137.4, 140.1, 148.7, 157. 157.6.
12	Fmoc-gAsp(OBzl)-Bsmoc	169	−21.59	89	1700 661.6323	2.3 (2H, s), 2.95 (2H, m), 3.7, (1H, m), 4.17 (1H, t), 4.39, (2H, d), 4.9 (1H, s), 5.1, (3H, m), 7.24- 7.8 (17 H, m)	43.8, 47.0, 63.1, 63.8, 69.4, 69.8, 119.6, 119.8, 124.1, 126.2, 127.1, 129.0, 132.3, 137.47, 140.2, 148.7, 157.6, 157.8, 171.
13	Fmoc-gCys(SBzl)-Bsmoc	184	−8.98	88	1712 649.7434	1.93 (2H, m), 3.83 (1H, br s), 4.2 (1H, t), 4.4 (2H, d), 5.1, (1H, s), 5.2 2H, s), 5.98 (1H, br s), 6.3 (1H, br s), 7.1- 7.8, (17H, m)	37.1, 38.8, 47.1, 67.4, 69.7, 118.9, 119.7, 119.8, 124.0, 126.2, 127.22, 128.2, 132.2, 137.8, 141.5, 148.7, 158.6, 158.7

Table III. Synthesis of retro–inverso peptides

Entry	Substrate	M.P. (°C)	$[\alpha]^{25}_{D}$ (c = 1, DMSO)	Yield (%)	I.R (cm^{-1})	Mass	^{1}H NMR	^{13}C NMR
a	Fmoc-gAla-rLeu-Boc	190	+102	40	1712	518.6823	0.82 (6H, m), 1.2 (3H, d), 1.34 (9H, s), 1.4 (2H, m), 4.08 (1H, t), 4.2 (2H, d), 5.1 (1H, d), 6.1 (1H, d), 6.35 (1H, d), 7.31-7.87	22.8, 24.7, 24.8, 27.0, 44.2, 47.1, 53.0, 59.7, 69.3, 78.9, 119.9, 124.2, 126.3, 127.1, 141.6, 148. 8, 155.4, 157.5, 172.
b	Fmoc-gLeu-rAsp(OBzl)-Boc	198	+133	35	1702	652.7182	0.82 (6H, m), 1.34 (9H, s), 1.4 (2H, m), 2.5 (1H, m), 3.0-3.3 (1H, m), 5.1 (2H, m), 5.2 (1H, d), 6.2 (1H, d), 6.38 (1H, d), 7.24-7.87 (13H, m)	21.6, 23.9, 28.3, 39.4, 44.3, 47.0, 50.2, 54.6, 62.6, 69.6, 78.9, 119.9, 124.1, 126.7, 128.2, 128.4, 140.6, 142.9, 148.8, 154.0, 157.0, 170.7, 172.
c	Fmoc-gIle-rPhe-Z	182	+98	40	1715	625.8234	0.91 (6H, m), 1.14 (1H, m), 1.54 (2H, m), 3.09 (2H,d), 3.66 (2H, m), 4.19 (1H, t), 4.40 (2H, d), 5.1 (2H, s), 5. 96 (1H, br s), 6.2 (1H, br s), 7.10- 7.87 (18 H, m).	12.2, 12.6, 27.3, 35.6, 37.7, 47.1, 51.3, 62.8, 65.9, 69.5, 119.9, 124.1, 126.3, 126.3, 128.8, 127.3, 127.4, 128.9, 130.4, 128.7, 136.4, 141.6, 148.8, 155.4, 156.0, 172.0.
d	Fmoc-gVal-rAla-Z	193	+122	44	1698	538.6437	0.93 (6H, t), 1.16 (3H, d), 2.03 (1H, m), 3.67 (1H, m), 3.81 (1H, m), 4.18 (1H, t), 4.40 (2H, d), 5.1 (2H, s), 5.98 (1H, br s), 6.4 (1H, br s), 7.1-7.87 (13 H, m).	14.6, 20.1, 30.9, 47.6, 57.7, 61.8, 66.6, 69.5, 119.9, 124.1, 127.3, 127.4, 127.7, 135.8, 141.5, 148.8, 155.4, 156.2, 174.0.

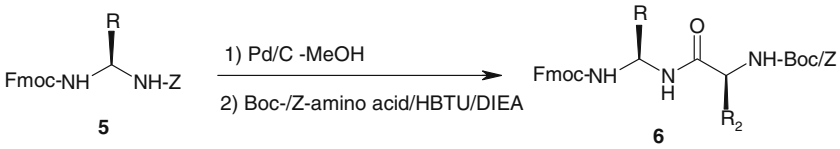
stirred at room temperature for 2–3 h. The reaction mixture was concentrated, the separated solid was recrystallized using ethyl acetate: hexane (4:6).

RESULTS AND DISCUSSION

Initially, Fmoc-amino acid azides in toluene was converted to its isocyanates. It was then used directly for the carbamate synthesis (Scheme 1). Thus, without the isolation of the isocyanate, one pot conversion of compound **2** with alcohol was attempted in the absence of any catalyst. Even after refluxing the solution for about 15 h, this proved to be an inefficient route that was resulted by low yields (25% for reaction with benzyl alcohol and 13% with *tert*-butyl alcohol). In an effort to improve the yield of the carbamate (Table I) while at the same time reducing the need for refluxing for long periods, we employed a reported procedure using Lewis acid catalyst for this reaction. Employing copper (I) chloride as a catalyst at r.t., even after stirring the reaction mixture for several hr, no improvement has been noticed over the non catalyzed thermal reaction, although this catalyst has been demonstrated to be useful in the synthesis of alkyl carbamates. (Duggan and Imagire et al., 1989). Ultimately, the alcoholysis of the isocyanate has been explored by utilizing MW irradiation.

In a typical reaction, a solution of Fmoc-amino acid azide in toluene in a closed teflon vessel was exposed to MW irradiation for 30 s. After cooling the solution to r.t., 1.2 equivalents of benzyl alcohol was added and exposure to MW irradiation was continued for 15 min with four intervals. Evaporation of the remaining solvent and recrystallization of the resulting residue has resulted in N-Fmoc-N^1-Boc-/Z-/Alloc-/Bsmoc-gem-diamines in 80–94% yield.

All the bisprotected *gem*-diamines made have been obtained in good yields as well as purity (Table II). They have been fully characterized by IR, ^{1}H and ^{13}C NMR and mass spectrometry. The protocol is then extended for the synthesis of N-Fmoc-N^1-Bsmoc- *gem*-diamines because the use of 1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc) group has been demonstrated to have added advantages over Fmoc group. For the synthesis of these gem-diamines, to the solution of isocyanates in toluene was added 1,1-dioxobenzo[*b*]thiophene-2-methanol (Bsm-OH) and exposed to MW irradiation for 15 min with four intervals. After the completion of the reaction, evaporation of the remaining solvent



Scheme 2.

and recrystallization of the residue has resulted in the isolation of the products in good yield (Table II).

The utility of N^{α}-Fmoc-N^{1}-Z-*gem*-diamines is demonstrated by the synthesis of four retro-inverso dipeptides. Briefly, the N-Fmoc-N^{1}-Z-*gem*-diamine is subjected to catalytic hydrogenation at r.t. using 10% Pd/C for 2 h and then the solution was filtered through celite. The resulting solution was evaporated in vacuo at 25 °C. It was directly coupled with Boc-/Z-amino acid employing HBTU to obtain the retro-inverso peptides (Scheme 2). All the peptides have been obtained as crystalline solids which were characterized by ^{1}H NMR, ^{13}C NMR and mass spectra. However, due to the poor solubility of N^{α}-Fmoc-N^{1}-Z-*gem*-diamines in methanol, the yields of the mono protected N^{α}-Fmoc-*gem*-diamines was not satisfactory (Table III).

CONCLUSION

It is now demonstrated that the alcoholysis of the isocyanates derived from Fmoc-amino acids can be carried out very efficiently and rapidly resulting in the isolation of the stable N-Fmoc-N^{1}-Z-/Boc-Alloc-/Bsmoc-*gem*-diamines in good yields and purity. The deprotection of Z group employing suitable conditions followed by coupling with N^{α}-protected amino acid result in the retro-inverso peptides, which is a an efficient approach.

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REFERENCES

Carpino, L.A., Beyermann, M., Wenschuh, H. and Bienert, M.: 1996, *Acc. Chem. Res.* 29, 268.

Carpino, L.A., Philbin, M., Ismail, M., Truran, G.A., Mansour, E.M.E., Iguchi, S., Ionescu, D., El-Faham, A., Riemer, C., Warrass, R. and Weiss, M.S: 1997, *J. Am. Chem. Soc.* 119, 9915.

Carpino, L.A., Philbin, M., Ismail, M., Truran, G.A., Mansour, E.M.E., Iguchi, S., Ionescu, D., El-Faham, A., Riemer, C and Warrass, R: 1999, *J. Org. Chem.* 64, 4324.

Chorev, M. and Goodman, M: 1993, *Acc. Chem. Res.* 26, 266.

Chorev, M., MacDonald, S.A. and Goodman, M: 1984, *J. Org. Chem.* 49, 821.

Claude, S., Marc-Andre, J., Cedrickx, G. and Melissa, A: 2005, *J. Org. Chem.* 70, 6118.

Cushman, M., Jurayj, J. and Moyer, J.D: 1990, *J. Org. Chem.* 55, 3186.

Duggan, M.E. and Imagire, J.S: 1989, *Synthesis* , 131.

Englund, E.A., Gopi, H.N. and Appella, D.H: 2004, *Org. Lett.* 6, 213.

Fletcher, M.D. and Campbell, M.M: 1998, *Chem. Rev.* 98, 763.

Helene, L. and Olivier, L: 2005, *Org. Lett.* 7, 4107.

Moutevelis-Minakakis, P. and Photaki, I: 1985, *J. Chem. Soc. Perkin Trans I.* , 2277.

Pallai, P.V., Richman, S., Struthers, R.S. and Goodman, M: 1983, *Int. J. Peptide Protein Res.* 21, 84.

Patil, B.S. and Sureshbabu, V.V: 2003, *Lett. Pept. Sci.* 10, 93.

Patil, B.S., Vasanthakumar, G.R. and Sureshbabu, V.V: 2003, *J. Org. Chem.* 68, 7274.

Scheibler, L., Chorev, M., Methods of Organic Chemistry: Synthesis of Peptides and peptidomimetics, M. Goodman, (ed), Houben-Weyl publishers, Vol 22a, (2002) 528.

Sureshbabu, V.V., Ananda, K. and Vasanthakumar, G-R.: 2000, *J. Chem. Soc. Perkin Trans I.* , 4328.

Ulrich, H.: 1996, *The Chemistry and Technonogy of Isocyanates*, pp. , Wiley, New York.

Vasanthakumar, G.R., Ananda, A. and Sureshbabu, V.V: 2002, *Indian. J. Chem.* 41B, 1733.